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ULTRASENSITIVE DETECTION OF CHEMICAL SUBSTANCES

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QUARTERLY REPORT

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During the past quarter, we have produced and cloned several hybridomas with antibody against AChE or AChE-DFP conjugate. One of these antibodies, designated V16-6B, has special interest. As expected this antibody binds the AChE-DFP conjugate effectively and shows a lowered cross reaction with AChE alone. The remarkable feature of this antibody is that it does not appear to bind DFP significantly. Figure 1 shows the protective effect V16-6B has in inhibiting the inactivation of AChE by decreasing amounts of DFP. Normally, DFP will inactivate AChE rapidly. However, if an agent such as an antibody is introduced which binds DFP then the reaction between AChE and DFP will be slowed. This data is very preliminary and requires a number of further control experiments. These will include more accurate quantization of the antibody so that molar ratios and equilibrium binding conditions can be better established and control conditions that include a protein such as non-reactive Goat IgG. This data does suggest, however, that V16-6B may be directed against an AChE site which changes determinant form or conformation when DFP binding takes place and that this site is probably not located at the active site of AChE.

That AChE should have a determinant site that changes its conformation when irreversibly bound to DFP is not surprising. It is known that AChE is an enzyme with a positive entropy of activation. A positive entropy of activation indicates strong structurally disorganizing events. It has been proposed that AChE carries a significant amount of structured H_2O molecules strongly associated with it and that during the enzyme reaction this organization is lost due to molecular changes in the enzyme. The loss of coupled molecules such as H_2O together with fundamental alterations in the AChE molecule itself would provide an antibody binding site which changes uniquely as a result of binding that occurs at the AChE active site. This data is very interesting and suggest possibilities of detecting a wide variety of potential toxins that

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inactivate AChE. This detection scheme based on conformational change at present has problems of crossreactivity. These problems make it impractical for ultrasensitive detection at this time, but through further selection of monoclonal antibodies crossreaction may be reduced. More important, is that antibodies such as this can be very useful in broad detection schemes where sensitivity is not as important as the variety of potential toxins able to be tested. In addition, the principle of utilizing antibody against a two state conformational change can be effective with toxins directed against other sensitive receptors. For example, the acetylcholine receptor (AChR) is subject to inactivation by a number of substances. It would be interesting to discover if conformational changes that could be detected by antibody accompanying these binding events.

In our proposal we outlined several areas of antibody research which could have a significant impact on detection systems. Our first problem was to make antibody to a specific preselected site on AChE. We have accomplished this task. The ability to make antibody to preselected sites on macromolecules is important in detection. Recently, Learner (1) and others have found that antibodies can be made against short peptide sequences that effectively bind to the same sequence when found in an altered conformation in an intact protein. Thus, a short polymer sequence may be used to direct antibody against a pre-determined polymer sequence. We are interested in developing these techniques for application in detection systems. We have begun collaborations with Dr. Terence Scallan to produce monoclonal antibodies against sterol carrier protein II. Dr. Scallan will provide sized peptide fragments from this protein for the

Niman, H.L., Houghten, R.A., Walker, L.E., Reisfeld, R.A., Wilson, I.A., Hogle, J.M. and Lerner, R.A. Proc. Nat'l. Acad. Sci. USA 80:4949-4953 (1983).

production of specific monoclonal antibodies. The development of these methods will give us an increased capability to produce monoclonal antibodies for use in detection schemes.

We have also initiated development of anti-idiotypic antibodies against two antibodies we have made against AChE and AChE-DFP conjugate. Anti-idiotypic antibodies can be used to reproduce structural features of a receptor or enzyme by internally imaging the first antibody. If we can make anti-idiotypic antibody which mimics a receptor site where toxin binds, then this antibody should be useful in detecting the toxin. In addition, since the antibody is an image of the receptor, it may have significant crossreactions with other toxins that would bind the receptor site.

We have now completed most of our work with Rivanol. This agent binds several proteins to polystyrene quite well. Figures 2,3, and 4 show the kinetics, temperature and ionic variation that contribute to this binding phenomena. We have tried analogs of Rivanol for binding, but our data is inconclusive at this time. We will continue this study in an effort to understand the mechanism of Rivanol binding.

In the course of our studies with Rivanol we found a bacterial species contaminating some of our buffers. This bacteria produced a non-filterable substance which also increased binding of protein. We have cloned this organism and found that it grows best at low temperatures, i.e., room temperature or below. We are interested in the substance this organism secretes which enhances protein binding to polystyrene. In the coming weeks we will determine if this substance has further use.

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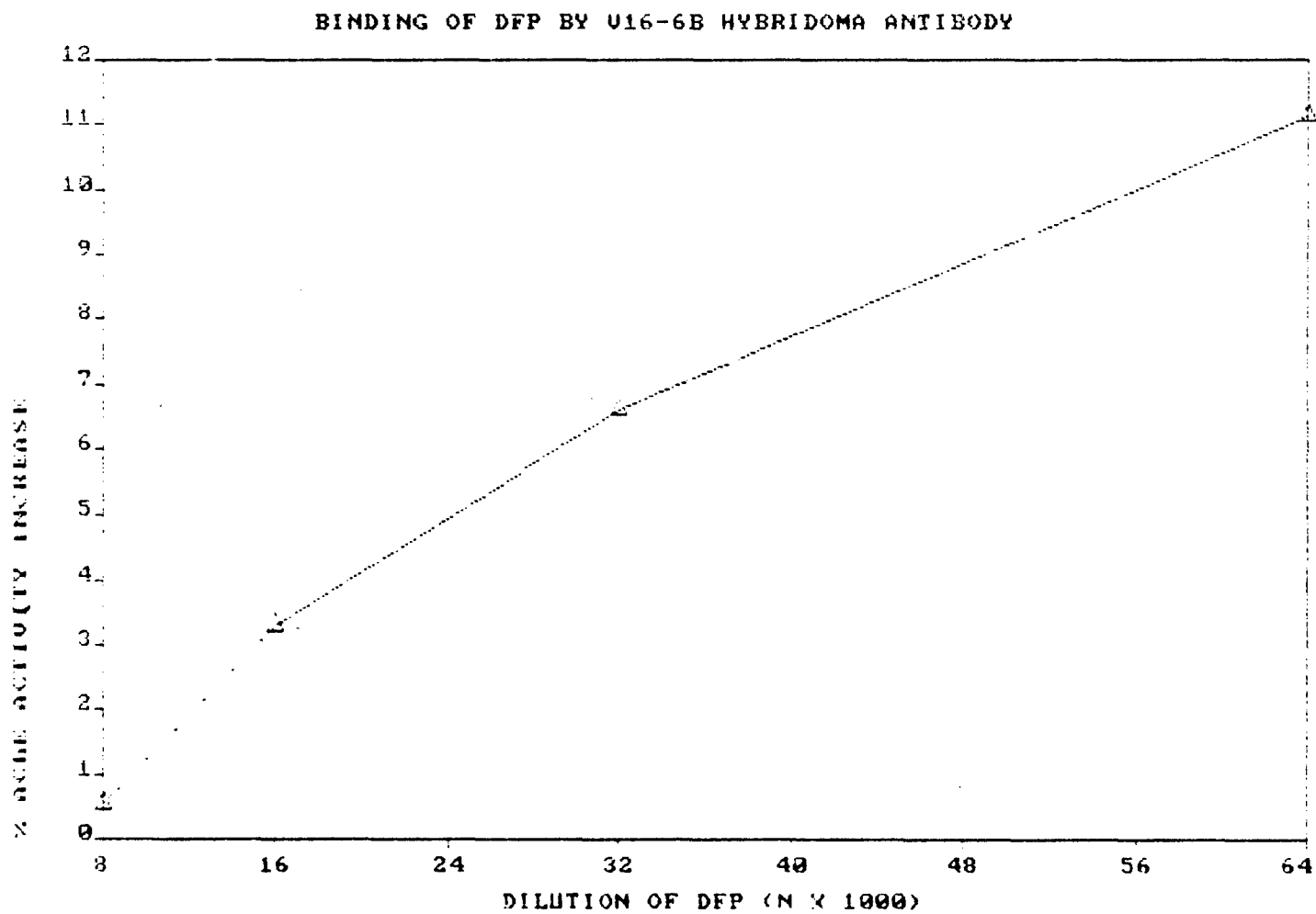


Figure 1: BINDING OF DFP BY V16-6B HYBRIDOMA ANTIBODY

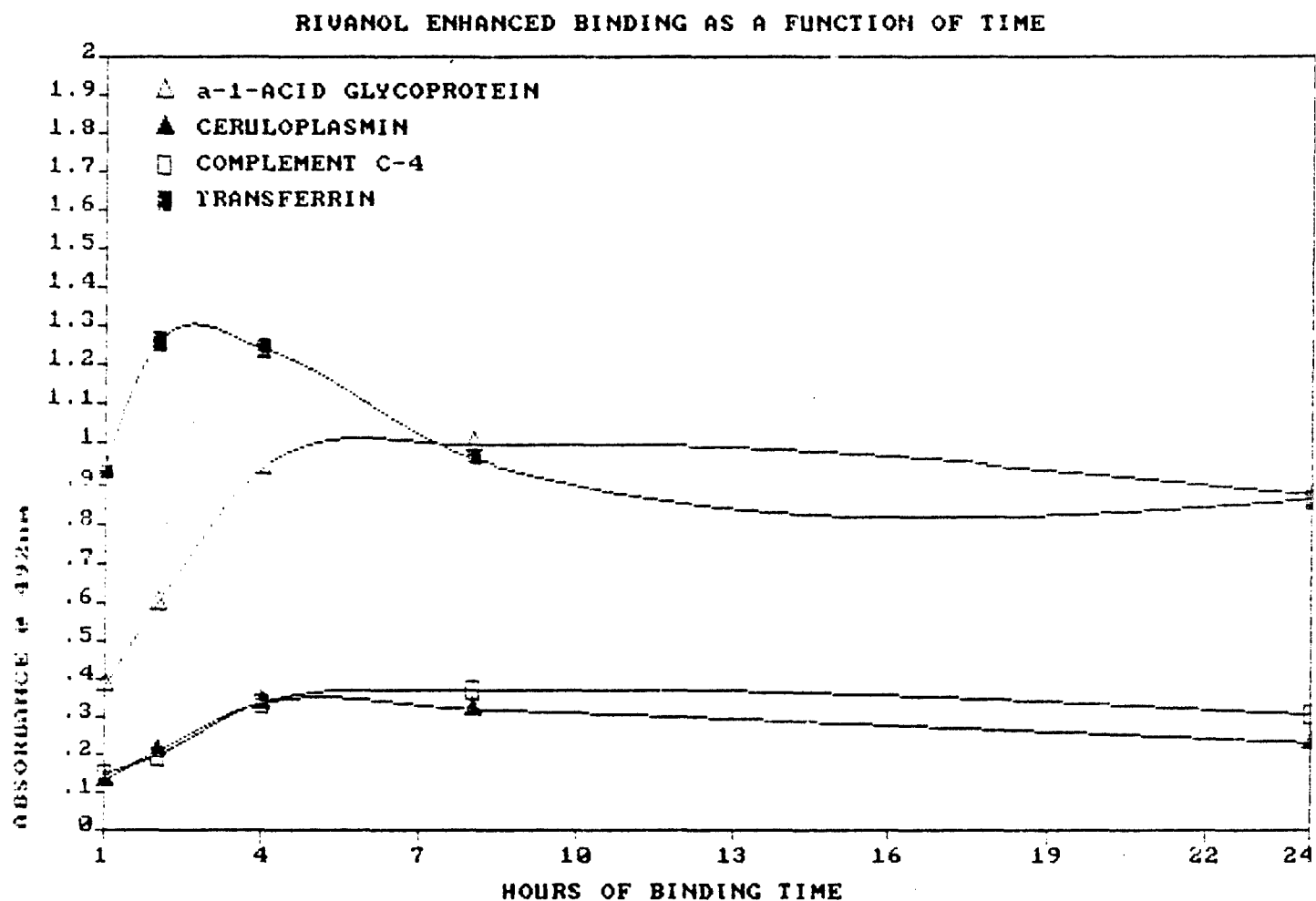


FIG. 2: Time study of select Human serum proteins in the presence of 1.5 % 15mM Rivanol. Saturation of solid phase is reached quite quickly and prolonged incubation is possibly detrimental to the reaction.

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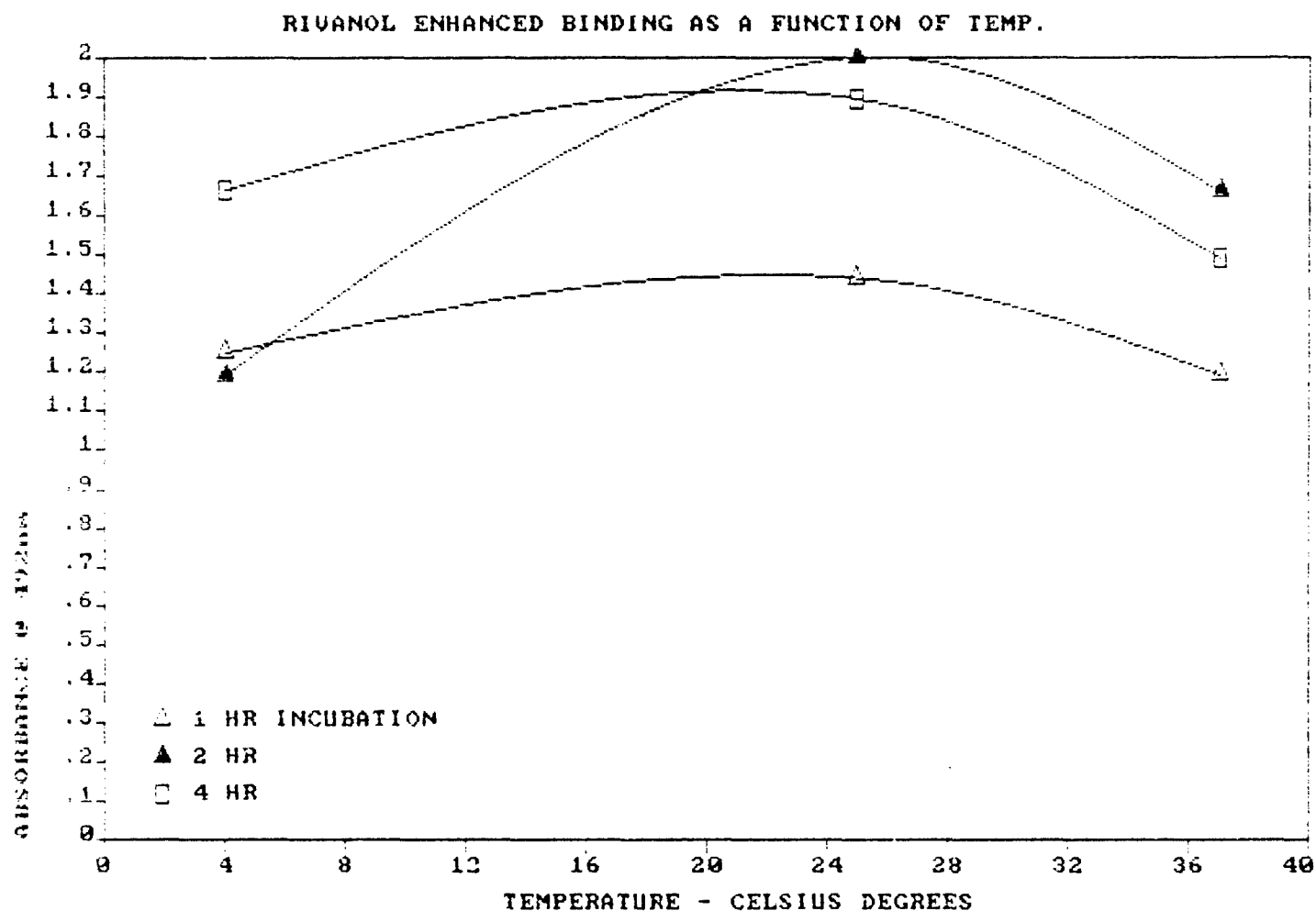


Fig. 3: Binding of Mouse IgG in the presence of Rivanol was examined at three commonly used temperatures i.e. 4, 25, 37 °C. For the times of interest the optimum temperature was about 25 °C or what is considered to be room temperature.

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EFFECTS OF IONIC STRENGTH ON BINDING WITH RIVANOL

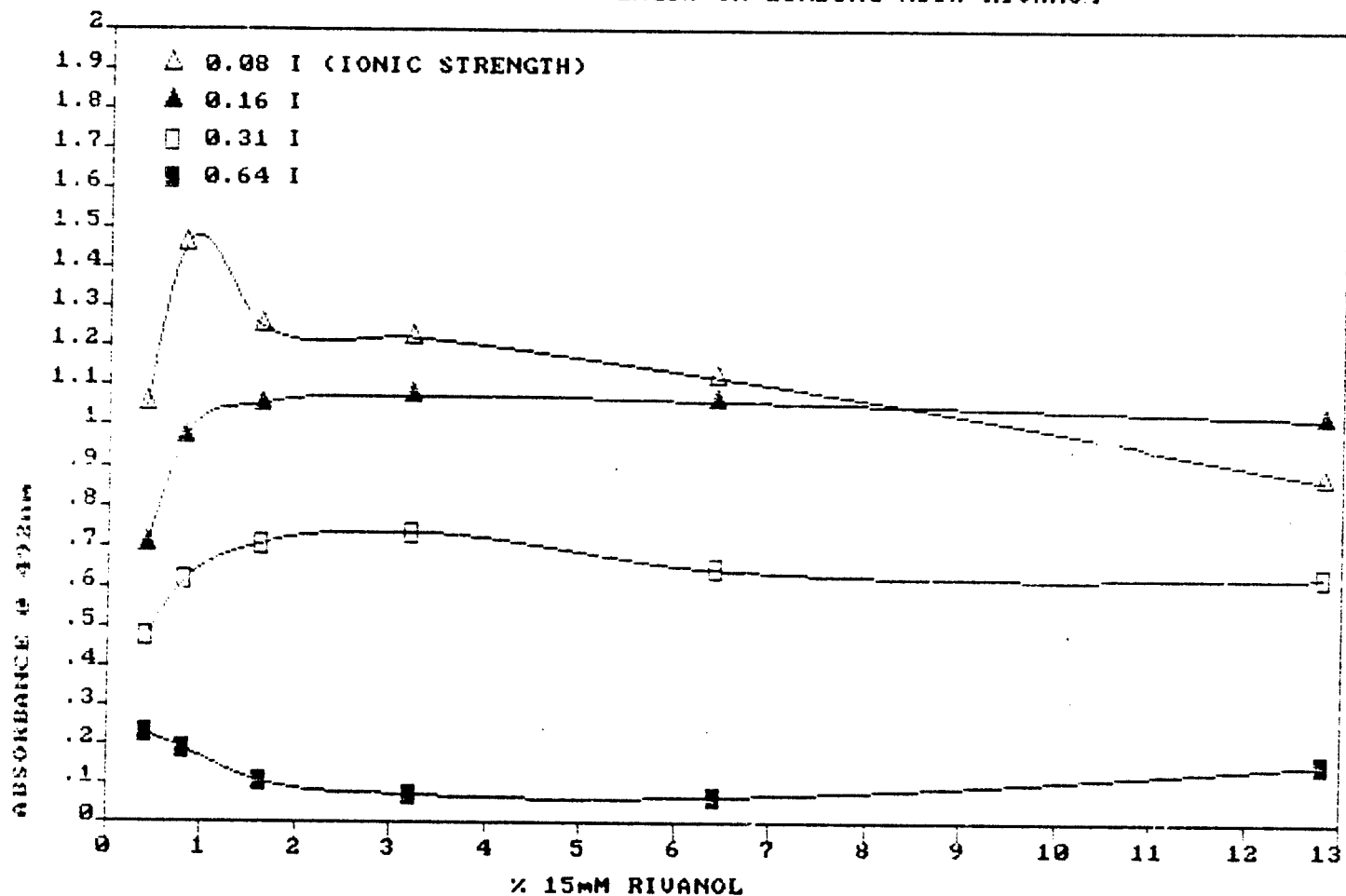


Fig. 4: Binding of Mouse IgG in the presence of 15mM rivanol decreases with increased ionic strength. Selected values for I were prepared by the addition of sodium chloride.

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